nature reviews gastroenterology & hepatology

https://doi.org/10.1038/s41575-025-01068-6

Review article

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Hepatic stellate cells: balancing homeostasis, hepatoprotection and fibrogenesis in health and disease

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Abstract

In the past decades, the pathogenic role of hepatic stellate cells (HSCs) in the development of liver fibrosis and its complications has been deeply characterized, rendering HSCs a primary target for antifibrotic therapies. By contrast, the beneficial roles of HSCs in liver homeostasis and liver disease are only beginning to emerge, revealing critical regulatory and fibrosis-independent functions in hepatic zonation, metabolism, injury, regeneration and non-parenchymal cell identity. Here, we review how HSC mediators, such as R-spondin 3, hepatocyte growth factor and bone morphogenetic proteins, regulate critical and homeostatic liver functions in health and disease via cognate receptors in hepatocytes, Kupffer cells and endothelial cells. We highlight how the balance shifts from protective towards fibropathogenic HSC mediators during the progression of chronic liver disease (CLD) and the impact of this shifted balance on patient outcomes. Notably, the protective roles of HSCs are not accounted for in current therapeutic concepts for CLD. We discuss the concept that reverting the HSC balance from fibrogenesis towards hepatoprotection might represent a novel holistic treatment approach to inhibit fibrogenesis and restore epithelial health in CLD simultaneously.

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Key points

- Besides serving as a primary site for retinoid storage, quiescent hepatic stellate cells (HSCs) fulfil key functions in liver homeostasis through communication with endothelial cells, Kupffer cells and hepatocytes.
- HSCs exert critical hepatocyte-regulatory functions, controlling hepatocyte gene expression, metabolism, death and proliferation, and thereby modulate acute and chronic liver disease in a fibrosis-independent manner.
- In the injured liver, activated HSCs represent the primary fibrogenic cell type, promoting the development of liver fibrosis, cirrhosis, portal hypertension and hepatocarcinogenesis.
- During liver disease progression, homeostatic and hepatoprotective HSC functions, characteristic of the quiescent state, are lost with a gradual shift towards fibropathogenic functions.
- The shifting HSC balance from homeostasis and hepatoprotection towards fibropathogenic functions is not accounted for in current therapeutic concepts and could be leveraged to simultaneously inhibit fibrosis and restore liver homeostasis.

Introduction

Hepatic stellate cells (HSCs) constitute about 5–8% of cells in the healthy liver, where they reside in the space of Disse between the endothelial lining and hepatocytes[1]. In the healthy liver, HSCs maintain a quiescent state. Quiescent HSCs store large amounts of vitamin A in characteristic lipid droplets, a feature that led to their discovery by von Kupffer, Ito and Nemoto, and Wake[2–4]. Following liver injury, HSCs undergo a well-characterized activation process in which they differentiate from vitamin A-storing lipocyte-like cells into the predominant fibrogenic cell type of the liver[5–7]. As liver fibrosis constitutes the primary determinant of mortality in chronic liver disease (CLD)[8–12], the main focus of the field in the past decades has been on understanding and targeting fibrogenesis, including mechanisms that drive and maintain the activated extracellular matrix (ECM)-producing state of HSCs. Additional interest has been in how HSCs regulate inflammation, as it is tightly associated with the development of liver fibrosis[13,14]. By contrast, the homeostatic and beneficial roles of HSCs beyond the storage of vitamin A are only beginning to emerge as proper tools to selectively manipulate HSCs have been lacking for a long time[15–19]. Such beneficial roles must undoubtedly exist to justify the existence of HSCs in all higher species. Their anatomical position within the space of Disse renders HSCs a potential hub in cell–cell communication of the liver. Accordingly, HSCs are in direct contact with hepatocytes and endothelial cells through distinct inter-sinusoidal and subendothelial processes, respectively[20,21]. Furthermore, Kupffer cells, while residing in the hepatic sinusoid, project part of their body into the space of Disse and interact with HSCs via specific processes[22,23]. Based on these microanatomical connections, it has been proposed that HSCs and their neighbouring cells form a functional unit, termed 'stellon', with HSCs as a central hub connected to a large number of hepatocytes on one side and endothelial cells on the other side[21], with additional contacts by Kupffer cells. Besides contact-dependent interactions, this anatomical context also provides the basis for spatially restricted interactions between HSCs and their neighbours via locally secreted mediators. Accordingly, HSCs interact closely with neighbouring cells via soluble mediators, regulating key liver functions in health and disease[24,25]. Several of these soluble mediators seem to be locally restricted, befitting the definition of morphogens[26,27], a class of mediators with critical roles in patterning during development, homeostasis and disease processes in many organs, including the liver[28–30]. Accordingly, patterning — mostly referred to as 'zonation' in the context of the liver — regulates essential liver functions in health and disease[31,32] and seems to be regulated by HSC-derived mediators in collaboration with well-established signals from endothelial cells[25,33].

This Review seeks to comprehensively summarize HSC functions in health and disease, focusing on their newly uncovered homeostatic and protective roles in regulating hepatic architecture and functions, including metabolism, injury and regeneration, in health and disease. Evolving evidence suggests that the role of HSCs changes during the progression of CLD and the development of hepatocellular carcinoma (HCC), with a shift from HSC-mediated protection towards disease-promoting pathofibrogenic functions[25,34]. A holistic view that integrates the protective and pathogenic functions of HSCs will enhance our understanding of hepatic pathophysiology and expand therapeutic concepts beyond the purely pathofibrogenic role that HSCs have traditionally been associated with.

HCSs in liver homeostasis

HSCs as a hub for communication in the healthy liver

The large number of HSCs in the healthy liver suggests functions beyond liver repair, as fibrogenesis could alternatively be accomplished by a lower number of HSCs that rapidly expand and migrate following liver injury. The concept of non-fibrogenic functions is further supported by the finding that HSCs express a wide repertoire of ligands and receptors, rendering them one of the most interactive cell types in the liver[24,34–36]. Accordingly, soluble HSC mediators, also termed 'stellakines', were proposed to regulate interactions with their neighbouring cells in health and disease[24]. Studies in mice with HSC depletion and HSC-selective knockouts have confirmed important roles for HSCs in communication with hepatocytes, endothelial cells, and Kupffer cells and point towards regulatory and protective functions in hepatic homeostasis via multiple HSC-enriched mediators[25,33,34,37] (Fig. 1). Rather than regulating liver homeostasis in a hierarchical fashion, HSCs constitute central components of multicellular signalling circuits that control key aspects of liver architecture and function. Such multicellular units, composed of HSCs, endothelial cells, Kupffer cells and hepatocytes, might enable fine-tuning of the regulation of complex and often zonal liver functions related to metabolism, inflammation and host defence. Accordingly, HSCs not only engage in direct interactions with hepatocytes, endothelial cells and Kupffer cells[25,33,34,37] but also participate in signalling circuits in which HSCs and endothelial cells collaboratively regulate hepatocyte gene expression and function. Examples of such multicellular signalling circuits include the regulation of hepatocyte function, survival and proliferation by the WNT–RSPO3–β-catenin and hepatocyte growth factor (HGF)–MET pathways[25,34], reviewed in further detail in the following paragraphs. Contact-dependent communication between HSCs and their neighbouring cells is not as well understood as communication via soluble mediators. Contact likely enables HSCs to sense key parameters in their environment and act as an interface between endothelial cells, hepatocytes and Kupffer cells, promoting intercellular signalling and transport[21]. Moreover, contact might

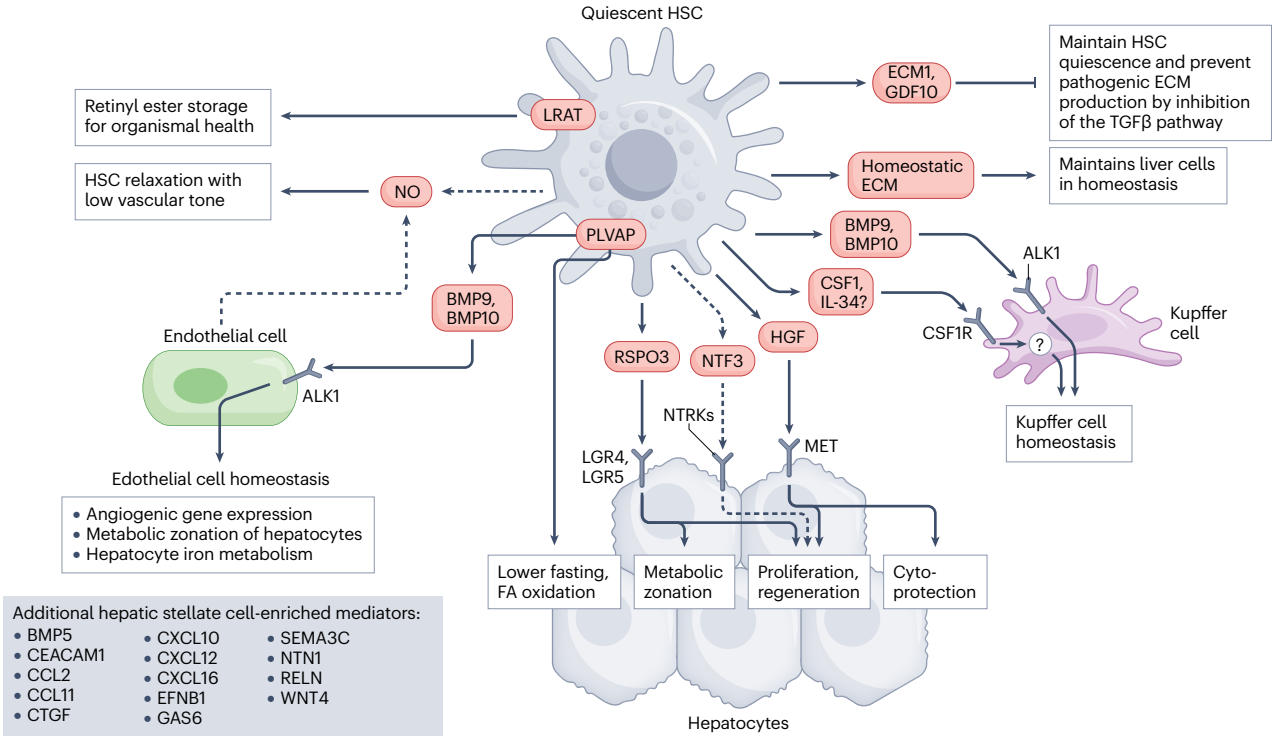

Fig. 1 | Protective HSC interactions in homeostasis and liver disease. Interactions of hepatic stellate cells (HSCs) with hepatocytes, mediated by HSC-derived R-spondin 3 (RSPO3), hepatocyte growth factor (HGF) and neurotrophin 3 (NTF3), control proper liver zonation and homeostatic regeneration and protect from injury. Interactions of HSCs with Kupffer cells via HSC-derived bone morphogenetic protein 9 (BMP9) and BMP10, CSF1, and IL-34 promote Kupffer cell homeostasis. The expression of homeostatic extracellular matrix (ECM) maintains a physiological environment for all liver cells and contributes to homeostasis. The expression of ECM1 and growth/differentiation factor 10 (GDF10) prevent transforming growth factor-β (TGFβ) activation and spontaneous HSC activation. Interactions of HSCs with endothelial cells via HSC-derived BMP9 and BMP10 regulate angiogenic gene expression in endothelial cells, thereby contributing to the regulation of liver zonation. Nitrix oxide (NO) from HSCs and endothelial cells acts on HSCs to maintain a low vascular tone. Expression of lecithin retinol acyltransferase (LRAT) enables HSCs to maintain high retinoid stores for organismal health. Together, these HSC interactions ensure homeostasis with proper metabolism (executed by hepatocytes) and shielding from gut-derived bacteria (via Kupffer cells). The described protective HSC interactions are gradually lost during chronic liver disease development. ALK1, activin receptor-like kinase 1; CCL, CC-chemokine ligand; CSF1R, CSF1 receptor; CTGF, connective tissue growth factor; CXCL, CXC-chemokine ligand; ECM1, extracellular matrix protein 1; EFNB1, ephrin B1; FA, fatty acid; GAS6, growth arrest-specific protein 6; LGR, leucine-rich repeat-containing G-protein coupled receptor; NTRK, NTF3 receptor neurotrophic receptor tyrosine kinase; NTN1, netrin 1; PLVAP, plasmalemma vesicle-associated protein; RELN, reelin; SEMA3C, semaphorin 3C.

serve as an amplifier of cell–cell communication as demonstrated for fibroblast–macrophage interactions in other settings[38,39]. In addition to cell–cell communication via subendothelial and inter-sinusoidal processes, HSCs exert pericyte-like functions, regulating the sinusoidal tone and possibly the exchange of fluids, solutes and particles between the sinusoidal lumen and the space of Disse[15,21,40–42].

HSCs regulate hepatocyte zonation and metabolic functions

One of the most striking effects of HSC depletion, either via diphtheria toxin receptor activation or just EGFP death-inducing (JEDI) T cells, on mouse livers is a profound alteration of their zonation[25,33]. Zonation, the spatiotemporal division of labour between distinct hepatocyte compartments, follows well-established metabolite gradients and the dual blood supply of the liver, enabling complex sequential multistep metabolic processes, such as lipid, bile acid and glucose metabolism, in different zones with adaptation to temporal changes in metabolism postprandially or during starvation[32]. One of the top alterations in HSC-depleted mouse livers is the WNT–β-catenin pathway, a master regulator of liver zonation and function[43–48]. HSC-depleted livers phenocopy key aspects of mice with hepatocellular β-catenin deletion[44,49,50], including an altered metabolic zonation with a condensed CYP2E1, CYP1A2 and RGN-positive pericentral to mid-lobular zone and an expanded CYP2F2 and HAL-positive periportal zone[25]. Although previous studies have assigned the regulation of liver zonation to endothelial cell-expressed WNT ligands and R-spondins[51–55], findings in HSC-depleted mouse livers suggest that HSCs either control these endothelial zonation signals or that HSC-derived signals provide additional or permissive signals in this multicellular regulatory network of the liver. Indeed, we identified HSC-derived R-spondin 3 (RSPO3) as a critical regulator of liver zonation[25]. Similar to hepatocytes, HSCs also show zonated gene expression patterns, and RSPO3 is a prototypical HSC zonation marker, displaying the highest expression in pericentral HSCs and lowest expression in the periportal HSCs[25,56,57] (Fig. 2). Notably, mice with HSC-selective deletion of *Rspo3* almost fully reproduce the phenotype of HSC-depleted mice and hepatocellular β-catenin deletion in regards to liver zonation and the expression of WNT-regulated genes[25]. Furthermore, HSC-selective deletion of *Rspo3* in mice leads to similar alterations in lipid and bile acid metabolism as in

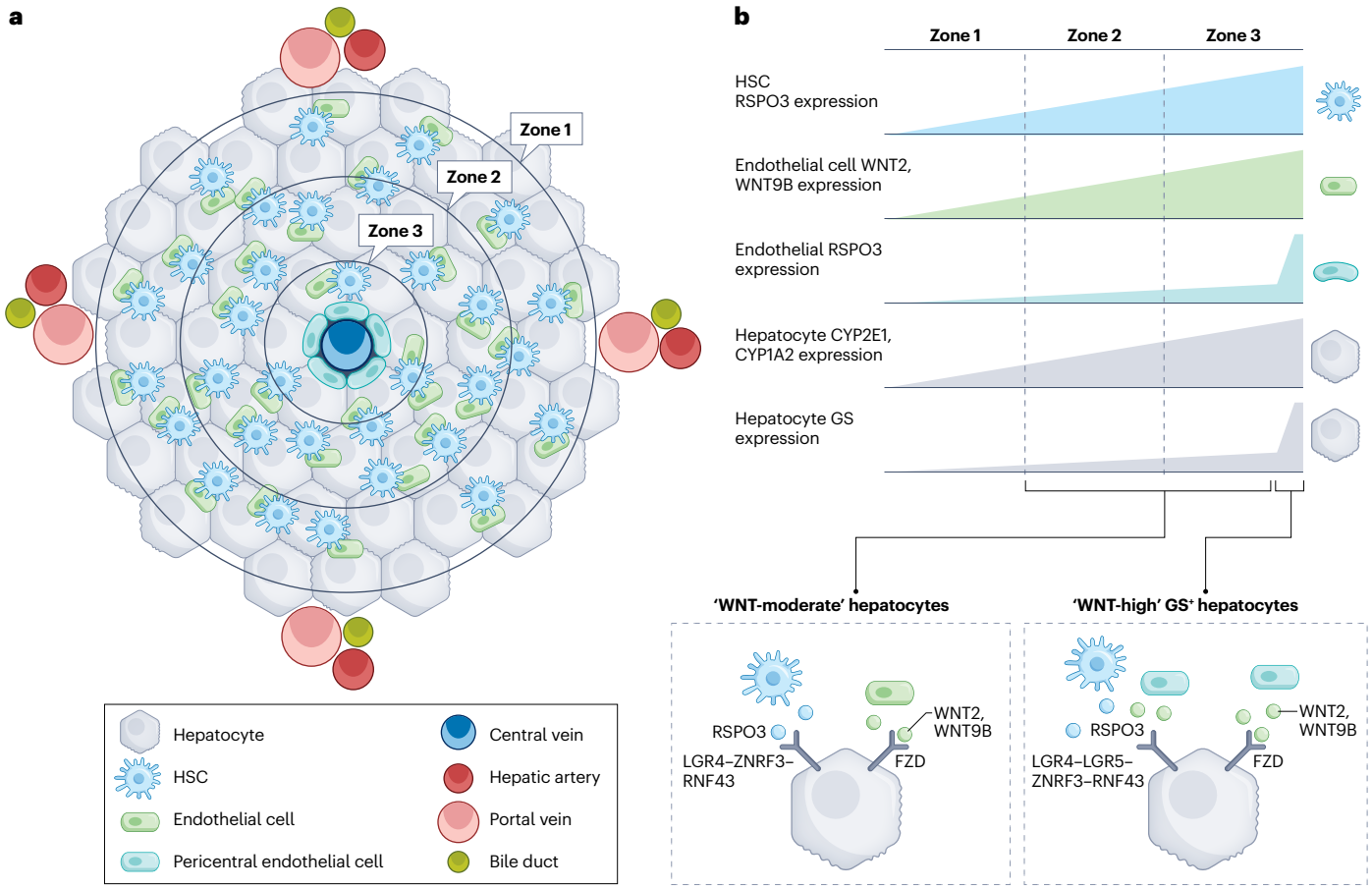

Fig. 2 | HSCs and liver zonation. Hepatic stellate cells (HSCs) and endothelial cells are in close proximity to each other and neighbouring hepatocytes throughout all zones (part **a**) but show differential expression of key regulators of β-catenin through different zones (part **b**). HSCs show a gradual decrease of R-spondin 3 (RSPO3) expression from zone 3 to zone 1. Endothelial cells display a gradual decrease of WNT2 and WNT9B expression from zone 3 to zone 1 but express RSPO3 only in the most pericentral layers. Accordingly, RSPO3 expression from endothelial cells induces high WNT activity, leading to glutamine synthetase (GS) expression in the most pericentral 'WNT-high' hepatocytes, whereas RSPO3 expression in HSCs is responsible for the expression of zonated genes such as *CYP2E1* and *CYP1A2* in the majority of 'moderate WNT' hepatocytes in zones 2 and 3. CYP, cytochrome P450; FZD, Frizzled; LGR, leucine-rich repeat-containing G-protein coupled receptor.

mice with hepatocellular β-catenin deletion[25,58]. RSPO3 is also expressed in endothelial cells, but the RSPO3 expression patterns between HSCs and endothelial cells differ substantially. RSPO3 is selectively expressed in a small number of the most pericentral endothelial cells at very high levels, whereas HSC expression is much broader and extends into the hepatic midzone, following a well-characterized pericentral to periportal expression gradient[25,56,57]. Consistent with its spatially restricted expression, endothelial cell-derived *Rspo3* does not regulate the majority of zones and WNT-regulated genes in the liver, with the exception of the most pericentral zones, where it regulates genes such as glutamine synthetase[25] in a few layers of 'WNT-high' hepatocytes[59]. Together, these findings suggest spatially distinct roles of HSCs and endothelial cells. HSC-derived RSPO3 triggers moderate WNT activity in large numbers of hepatocytes in zones 2 and 3, regulating key metabolic hepatocyte functions such as cytochrome P450 reactions as well as bile acid and cholesterol metabolism[25]. By contrast, endothelial cell-derived RSPO3 induces high WNT activity in a few layers of the most pericentral hepatocytes, maintaining high levels of glutamine synthetase expression precisely in the region where blood exits into the systemic circulation, preventing ammonia, a by-product of many metabolic pathways in the liver, from reaching other organs and inducing damage such as hepatic encephalopathy[25,60] (Fig. 2). RSPO3 is a ligand for LGR4 and LGR5, expressed throughout the liver or only in the pericentral area, respectively[46]. Mice with hepatocyte-specific deletion of LGR4 display similar zonal alterations, metabolite alterations and reduced liver size as mice with hepatocyte-specific deletion of β-catenin, HSC-depleted mice or mice with HSC-selective deletion of RSPO3 (refs. 33,43–46), suggesting that RSPO3–LGR4 represents a key ligand–receptor pair that mediates HSC-hepatocyte crosstalk and the regulation of liver zonation via β-catenin (Figs. 1 and 2). As pericentral hepatocytes express both LGR4 and LGR5, it is likely that both of these R-spondin receptors contribute to the 'WNT-high' state of these cells. R-spondins, via LGR4 and LGR5, potentiate WNT signals via the degradation of negative regulators of WNT signalling, ZNRF3 and RNF43, which downregulate Frizzled family members, the receptors for WNTs[61,62]. Hence, the activation of WNT signalling by R-spondins, LGR4 and LGR5, achieved by the

removal of inhibitory signals, requires the presence of WNT ligands such as WNT2 and WNT9B, which are mostly derived from endothelial cells[53,63] (Fig. 2). Parallels to this hepatic mesenchymal–epithelial cell crosstalk are found in the intestine, stomach, breast and hair follicles, where stromally expressed R-spondins regulate epithelial and stem cell niches (reviewed previously[64]).

Besides β-catenin-dependent zonation, HSCs might also regulate serum albumin levels, as shown in mice with JEDI-mediated HSC depletion[33]. However, the underlying mechanisms remain unclear as HSC-depleted mice did not show lower mRNA expression of albumin or other genes considered essential for synthetic function such as coagulation factors[33]. Moreover, HSC depletion via *Lrat*–Cre-induced diphtheria toxin receptor (iDTR) did not alter serum albumin levels (R.F.S., unpublished observations). Although this might be due to different degrees of depletion by JEDI and iDTR, further studies on the underlying mechanisms are needed as albumin synthesis is an essential function of the liver and affects patient outcomes[65]. Furthermore, HSCs might also regulate mitochondrial functions in hepatocytes[25], possibly via the RSPO3–β-catenin pathway. Consistent with this notion, β-catenin has been reported to affect mitochondrial biogenesis and oxidative phosphorylation in the liver and other organs[66–68].

HSCs also exert a role in the transport of fatty acids from the lumen to hepatocytes. HSCs abundantly express plasmalemma vesicle-associated protein (PLVAP) and control the hepatic uptake of non-esterified fatty acid during fasting in mice[42]. HSC-specific ablation of PLVAP suppresses hepatocyte fatty acid esterification to diacylglycerols and triacylglycerols along severe perturbations of hepatocyte programmes governing normal fatty acid handling, oxidation and ketogenesis, with suppression of genes involved in fatty acid utilization and ketogenesis such as *Acacb*, *Acox1*, *Acss3*, *Cyp4a10*, *Cyp4a14*, *Ehhadh*, *Hmgcs2* and *Pdk4*, suppressed genes for triglyceride storage such *Cidec*, *Elovl5*, *G0s2*, *Plin2* and *Plin5*, and upregulation of genes encoding for serum transport proteins such as albumin and transthyretin[42].

In summary, HSCs regulate the zonation and multiple metabolic functions of hepatocytes. However, further studies are needed to fully characterize the effects of HSCs on hepatocyte metabolic functions via β-catenin-dependent and β-catenin-independent pathways.

Regulation of hepatocyte proliferation and liver size by HSC-derived growth factors in homeostasis

The profoundly decreased size of the liver constitutes a second characteristic feature of HSC-depleted livers, which seems to be linked to the reduced expression of *Ccnd1* (encoding cyclin D1), a β-catenin target gene[25,33,69]. Mice with HSC-selective deletion of RSPO3 display a similar reduction of liver size and mid-lobular cyclin D1 expression as mice with HSC-depleted liver and hepatocyte-specific deletion of *Ctnnb1* (encoding β-catenin) or *Lgr4* deletion[25,33,46,70]. Thus, HSC-derived RSPO3, via its receptor LGR4 and downstream target β-catenin, not only seems to regulate zonation but also cyclin D1 expression and proliferation of mid-lobular hepatocytes, the proposed main cellular source for homeostatic liver regeneration[71]. In contrast to the strong effects of HSC-expressed RSPO3, HSC-expressed HGF, another HSC-enriched mediator that exerts potent effects on hepatocytes via its cognate receptor MET, seems to exert few effects on the healthy liver[34,72]. As the two major effects of HGF on hepatocytes are cytoprotection and proliferation[73–75], the consequences of HSC-specific deletion of *Hgf* in mice are more apparent in the setting of liver injury[25,34] (see the next section). The lack of strong effects of HSC-secreted HGF on homeostasis can also be attributed to the fact that only 70% of the liver HGF is produced by HSCs and about 30% by endothelial cells[34]. Furthermore, HGF is stored in the hepatic extracellular matrix, which can provide a buffer that can be filled both from HSC and endothelial cell HGF sources. Unlike RSPO3, HGF does not seem to be zonated or to exert spatially controlled effects[56]. Neurotrophins, including neurotrophin 3 (NTF3), represent additional HSC-enriched growth factors[33,76]. NTF3 overexpression in mice induces hepatocyte proliferation and cyclin D1 expression in HSC-depleted livers and corrects their decreased size[33]. The NTF3 receptor neurotrophic receptor tyrosine kinase 2 (NTRK2) shows the highest expression in mid-lobular zones, and pharmacological blockade of NTRK2 reduces hepatocyte proliferation induced by NTF3 (ref. 33). Together, these data suggest the NTF3–NTRK2 pathway as an additional driver of cyclin D1 expression and homeostatic hepatocyte regeneration besides RSPO3 (Fig. 1). However, further studies in mice with specific deletion of *Ntf3* in HSCs and deletion of NTF3 receptors in hepatocytes are needed to confirm this role. NTF3 also acts on HSCs via its receptor NTRK3, promoting HSC activation through an autocrine loop involving NTKR3 (ref. 77), suggesting additional cellular targets, including HSCs. There is a large number of HSC-enriched growth factors and mediators, such as bone morphogenetic protein 5 (BMP5), connective tissue growth factor, epiregulin, nerve growth factor and fibroblast growth factors (FGFs) FGF12 and FGF2, whose function in adult liver homeostasis remain unknown.

Regulation of Kupffer cell and endothelial cell homeostasis and HSC quiescence

Several HSC-secreted cytokines, also termed 'stellakines', were suggested to mediate interactions of HSCs with Kupffer cells and endothelial cells[24]. Functional investigations in mice have revealed that interactions of HSC-derived BMP9 (encoded by *GDF2*) and BMP10 with the Kupffer cell-expressed TGFβ receptor family member activin receptor-like kinase 1 (ALK1) are crucial for maintaining the Kupffer cell pool (Fig. 1). Accordingly, Kupffer cell-selective deletion of *Alk1* (refs. 78,79) or HSC-selective knockout of *Bmp9* and *Bmp10* in mice triggers a reduction of liver macrophages by replacing the resident Kupffer cells with monocyte-derived macrophages[37]. Moreover, HSCs are primary sources for two macrophage growth factors, CSF1 and IL-34 (ref. 22), which might additionally contribute to the maintenance of liver macrophages. It has been suggested that fibroblasts and macrophages form a stable two-cell circuit to control each other's density by exchanging growth factors, such as CSF1, and that physical contact enhances this growth factor exchange[38]. Moreover, contact between profibrotic macrophages and fibroblasts contributes to fibroblast activation via αvβ3 integrin expression on macrophages and Piezo1 expression on fibroblasts and has been proposed to 'kickstart' fibroblast activation[39]. Thus, it is likely that direct contact between HSCs and macrophages enhances the bidirectional crosstalk between both cell populations in homeostasis and disease.

BMP9 and BMP10 are also key mediators of HSC–endothelial cell communication (Fig. 1). Endothelial cells maintain HSC quiescence and the capillarization of endothelial cell in the diseased state is permissive for HSC activation[80]. Mice with HSC-specific double knockout of *Bmp9* and *Bm10* display alterations of endothelial cell function, leading to loss of master regulators GATA4 and MAF as well as capillarization, which in turn induces fibrosis, likely due to the loss of negative signals from endothelial cells on HSCs[37]. This finding is consistent with a critical role for the BMP9 and BMP10 receptor ALK1 in maintaining endothelial cell health[81]. Furthermore, HSC-secreted BMP9 and BMP10 regulated the

expression of angiocrine factors WNT2, WNT9B and RSPO3, leading to altered zonation of the liver as well as focal iron overload[37]. However, despite strongly decreased BMP9 and BMP10 expression, livers from HSC-depleted adult mice displayed only weak or no alterations of endothelial cell genes *Gata4*, *Bmp2*, *Bmp6*, *Wnt2* and *Wnt9b*[25]. Hence, some effects of HSC-derived BMP9 and BMP10 on endothelial cells and the subsequent effect on endothelial cell-mediated hepatocyte zonation might occur during liver development, consistent with the critical developmental functions of the BMP pathway[26]. It is likely that, besides BMP9 and BMP10, additional HSC-secreted mediators affect endothelial cells or are part of a bidirectional endothelial cell–HSC communication.

Finally, HSCs must be kept in a quiescent state to maintain homeostasis and prevent spontaneous fibrosis in the healthy liver. TGFβ, the most potent HSC-activating cytokine, is not only produced upon demand but, in its latent inactive form, is also bound to ECM components such as fibrillin, proteoglycans and fibronectin[82]. In homeostasis, the spontaneous activation of latent TGFβ is tightly controlled to prevent the erroneous development of liver fibrosis: ECM1, expressed in hepatocytes and quiescent HSCs, is a currently identified key player in the machinery that regulates the activation of latent TGFβ and thereby prevents spontaneous HSC activation[83,84]. Although this role has been ascribed to hepatocyte-expressed ECM1, it is possible that HSC-expressed ECM1 acts locally to specifically keep latent TGFβ in proximity to HSCs in an inactive state (Fig. 1). Secretion of growth/differentiation factor 10 (GDF10), a member of the BMP family, by HSCs inhibits the TGFβ–SMAD2/3 signalling pathway and maintains HSC in a quiescent state via an autocrine signalling loop that activates the inhibitory SMAD1/5/8–SMAD7 pathway[85]. Moreover, the above-described HSC–endothelial cell–Kupffer cell multicellular signalling circuits keep HSCs in a quiescent state via additional signals. Interfering with this regulating circuit, for example, via HSC-specific double knockout of *Gdf2* (encoding BMP9) and *Bmp10* in mice, not only affects Kupffer cell and endothelial cell homeostasis but also induces HSC activation and liver fibrosis[37]. On a transcriptional level, transcription factor LHX2 is important in maintaining HSC identity and quiescence[34,86,87], but it remains to be determined how LHX2 expression and activity are regulated in HSCs. Additional HSC mediators and transcription factors besides ECM1, GDF10, BMP9, BMP10 and LHX2 likely contribute to maintaining HSC quiescence and homeostasis.

The storage of retinyl esters by HSCs

HSCs are endowed with a high capacity for storing retinoids, accumulating as much as 50–80% of the body's retinoids in their characteristic lipid droplets in the form of retinyl esters[88–91]. Lecithin retinol acyltransferase (LRAT) is the sole enzyme that catalyses all-trans-retinol esterification and is highly enriched in HSCs[6,92,93]. The storage of retinoids within HSCs provides the body with a healthy reserve lasting for several weeks or months, thereby preventing hypovitaminosis A[94] (Fig. 1). Retinyl ester storage is zonated, and the highest levels are found in periportal HSCs, reflecting the gradient of vitamin A that is delivered from food sources via the portal vein[15,21,95,96]. As retinyl esters represent an inert storage form of retinoids, retinyl ester-containing lipid droplets might serve as a buffer to prevent unwanted adverse effects of retinoids and toxic spikes after vitamin A ingestion[94]. The liver is the first organ to be hit by portally delivered nutrients from the intestine; HSCs might therefore represent a first line of defence, protecting hepatocytes from excessive vitamin A exposure. Such a protective function has been suggested for polar species, which accumulate unusually large amounts of retinoids as scarce top predators in their ecosystem[97]. In support of this hypothesis, LRAT-deficient mice displayed increased expression of retinoid-regulated genes *Cyp26a1*, *Cdkn1a* (coding for p21) and *Rarb* in their livers[92,98,99]. *Lrat* expression decreases with HSC activation[92,93], which might result in a reduced ability to buffer potentially toxic levels of retinoids. However, as both hypovitaminosis A and hypervitaminosis A can be toxic to the liver, HSC-expressed LRAT and retinoid storage could be both protective or disease promoting in a context-dependent manner.

Homeostatic ECM production by HSCs

It is well established that homeostatic ECM is a gatekeeper of tissue health and function that keeps cells in a differentiated and properly organized state in various organs[100]. Although excessive deposition of ECM is a key feature of the fibrotic liver, the normal liver also contains a characteristic ECM, constituting about 0.5% of its weight[15,101]. The majority of the healthy liver's ECM is either in its capsule or surrounding vessels and bile ducts in the portal triad and the central vein area[102]. Although sinusoids contain less ECM than perivascular or periductular areas, a healthy homeostatic ECM is important to keep cells surrounding the sinusoids in a physiological state (Fig. 1). Accordingly, published studies and single-cell RNA-sequencing (scRNA-seq) databases demonstrate that HSCs, endothelial cells and hepatocytes express ECM proteins in the healthy liver of mice and human[15,78,103–105]. Whereas endothelial cells predominantly secrete type IV collagens, typically found in basement membranes, and hepatocytes secrete fibronectin, quiescent HSCs express a wide range of ECM members, including fibrillar collagens such as type I, type III and type V, type XIV collagen, laminin, decorin and biglycan as well as type IV collagen, albeit at lower levels than endothelial cells[15,78,103–105]. The specific role of HSC-secreted ECM components in liver homeostasis remains currently unknown and needs to be tested via HSC-specific knockout approaches in young and aged mice. It is likely that HSCs, together with ECM provided by other cells, such as endothelial cells and hepatocytes, shape the hepatic extracellular environment that maintains liver homeostasis.

Protective HSC functions in the diseased liver

The protective roles of HSCs in acute liver disease and CLD are only beginning to be understood. There is emerging evidence that HSCs are not simply a minority cell population with specialized functions, such as vitamin A metabolism and fibrogenesis, but that they regulate key aspects of hepatocyte biology such as metabolism, injury and regeneration as well as non-parenchymal cell biology (Fig. 1), thereby affecting the progression of CLD and outcomes. Notably, the continuous loss of many protective HSC mediators during CLD (Fig. 1) might contribute to disease progression as well as the altered zonation that is characteristic of CLD[106–109]. Although the contribution of protective HSC-secreted mediators, such as RSPO3, HGF, BMP9 and BMP10, reviewed below, has been functionally established, the effect of many other HSC-enriched mediators (other HSC-enriched BMPs such as BMP5; collectins such as COLEC10 and COLEC11; PVLAP and RELN) on liver disease remains elusive.

Modulation of hepatocyte metabolism, systemic metabolism and steatotic liver disease by HSCs

Hepatocytes represent the central functional unit of the liver, exerting essential roles in lipid, protein, carbohydrate, bile acid and xenobiotic metabolism, which often fail during the development and progression of steatotic liver disease (SLD) and other forms of CLD[110]. Hepatocytes

are supported by other cells to execute these metabolic functions efficiently. Liver sinusoidal endothelial cells ensure the transport of incoming and outgoing metabolites[111], cholangiocytes connect hepatocytes to the biliary tree for biliary excretion of metabolites[112] and Kupffer cells function as a firewall for gut-derived bacteria[113]. Bidirectional communication of hepatocytes with other liver cells, as well as proper zonation, are essential to main hepatic metabolism[32] and their failure might drive or exacerbate SLD, including metabolic dysfunction-associated SLD (MASLD) or alcohol-associated liver disease (ALD).

The central role of HSCs in controlling the metabolic zonation of hepatocytes via RSPO3 influences the development of MASLD. The main effects of HSC-expressed RSPO3 and its downstream target in hepatocytes, β-catenin, are on lipid, bile acid and cholesterol metabolism as well as on the activity of cytochrome P450 oxidase (CYP450) family members and mitochondrial function[25,48,66,67,114]. The high expression of RSPO3 in quiescent HSC, which decreases during MASLD progression in mice and patients, represents a protective factor as apparent by the exacerbation of MASLD with increased steatosis, liver injury and fibrosis in mice with HSC-specific loss of *Rspo3* (ref. 25). Moreover, mice with HSC-selective *Rspo3* deletion display increased MASLD-associated HCC development[25]. Conversely, overexpression of *Rspo3* ameliorates hepatic steatosis in the *ob/ob* mouse model of MASLD[115]. A similar protective role for RSPO3 is seen in patients with MASLD, as demonstrated by an inverse correlation of *RSPO3* expression with mortality and liver-related events[25]. The downregulation of RSPO3 in MASLD is likely mediated by TGFβ, which decreases RSPO3 expression in mouse and human HSCs by approximately 75%[25]. Like hepatocyte knockout of *Ctnnb1* or *Lgr4* and *Lgr5* (refs. 58,116), HSC-specific knockout of *Rspo3* leads to pericentral accumulation of triglycerides and increased hepatic bile acid levels in mice[25]. Whether the regulation of targets such as CYP2E1 by the RSPO3–LGR4–β-catenin pathway contributes to the pathophysiology of MASLD remains to be determined as CYP2E1 not only carries out homeostatic functions, including lipid metabolism, but also contributes to lipid peroxide formation[117]. The role of the RSPO3–β-catenin pathway in mitochondrial biogenesis and function[25,66,67] and its effects on MASLD deserve further studies. Interestingly, hepatic overexpression of *Rspo3* improves systemic metabolism with improved insulin resistance and prevention of body weight gain[115]. In line with the key role of RSPO3 in hepatic and systemic metabolism, hepatic RSPO3 expression increases after feeding in lean mice but this upregulation is lost in obesity[115]. The effects of HSC-derived RSPO3 on systemic metabolism remain to be confirmed in mice with HSC-specific deletion of *Rspo3*.

Like in MASLD, the expression of *RSPO3* decreases in HSCs from patients with ALD[25]. Notably, HSC-specific deletion of *Rspo3* exacerbates ALD in mice, causing increases in steatosis and injury, similar to the exacerbated ALD phenotype in mice with hepatocellular β-catenin deletion[67,118]. Decreased activity of the RSPO3–β-catenin pathway might contribute to an exacerbation of ALD via the decreased expression of aldehyde dehydrogenase 2, which converts acetaldehyde to acetic acid, the altered liver zonation causing increased hepatic triglycerides and bile acids as well as the aforementioned alterations in mitochondrial function[25,66,67]. In patients with ALD, *RSPO3* expression is inversely correlated with mortality[25].

Together, these data suggest that HSC-expressed RSPO3 is a protective factor and a potential therapeutic target in SLD. How the aforementioned role of HSCs in the transport of non-esterified fatty acids via PLVAP, which has been demonstrated in fasting mice[42], affects the progression of MASLD and ALD diseases remains elusive. RSPO3 likely synergizes with additional HSC mediators directly regulating hepatocyte metabolisms, such as PLVAP, or HSC mediators that directly or indirectly regulate other aspects of hepatocyte biology, such as HGF, BMP9, BMP10 and NTF3, to protect from SLD.

Modulation of hepatocyte death by HSCs

Hepatocyte death is a central feature of CLD[119–121]. Different forms of cell death, including apoptosis, necroptosis, ferroptosis and necrosis, drive the progression towards fibrosis and HCC in CLD[119–122]. Depletion of HSCs via *Lrat*–Cre-driven iDTR or *Gfap*-driven thymidine kinase has revealed a role for HSCs in the regulation of liver injury in mice[25,34,123]. HSCs regulate hepatocyte death via multiple ligand–receptor systems. HGF represents the most potent HSC mediator, protecting against multiple forms of cell death. HSC-selective deletion of *Hgf* increases liver injury induced by carbon tetrachloride (CCl_4) and MASLD in mice[25,34], phenocopying the effects of hepatocyte-selective loss of its receptor *Met*[72,124,125]. The reduction of liver injury by HSC-derived HGF protects the liver from the development of fibrosis and increases injury-driven regeneration; together, these injury responses have a key role in promoting HCC development as seen in mice with HSC-selective *Hgf* deletion[34]. The expression of HGF decreases with HSC activation but is compensated by the expanded number of HSCs in the chronically injured liver[34]. RSPO3 represents another key HSC mediator that modulates hepatocyte death. However, the role of RSPO3 lies in positively regulating the expression of CYP2E1, CYP1A2 and other CYP450 enzymes, which metabolize a wide range of toxins such as acetaminophen and CCl_4 (ref. 25). Although CYP450-mediated toxin metabolization might be beneficial for overall health and in most physiological settings[126,127], the upregulation of CYP450 enzymes via RSPO3–β-catenin might increase hepatocyte death in mice after ingestion of large amounts of man-made toxins, such as acetaminophen and CCl_4, through the local generation of toxic metabolites, contrasting the hepatoprotective role that RSPO3 exerts in ALD and MASLD[25]. Similar to RSPO3, LRAT seems to also regulate CYP450 enzymes and might exert beneficial or detrimental effects[128,129]. Although LRAT promotes the elimination of toxins like bisphenol A, it also aggravates thioacetamide-induced liver injury in mice[128,129]. However, further studies are needed to confirm that alterations in CYP450-mediated hepatic xenobiotic metabolism and liver injury are indeed caused by LRAT expressed in HSCs, for example, by HSC-specific conditional deletion strategies. In summary, HSC-derived mediators seem to be primarily hepatoprotective but some mediators such as RSPO3 and LRAT may exert context-dependent effects as their effects on xenobiotic metabolism, promoting organismal health, might be accompanied by local liver toxicity in case of the ingestion of large amounts of man-made toxins.

Promotion of liver regeneration by HSCs

The liver's astounding regenerative ability sets it apart from most other organs. Regeneration occurs after acute and chronic injury, maintaining functional liver mass and ensuring survival[130]. Liver regeneration is controlled by a wide range of pathways[130]. Activation of MET and EGFR on hepatocytes exerts potent mitogenic effects, whereas most other pathways are permissive and only delay liver regeneration if bypassed[130]. Depletion of HSCs via gliotoxin, *Gfap*-driven thymidine kinase or *Lrat*–Cre-driven iDTR strongly impairs liver regeneration in the early stages after 70% partial hepatectomy but leads to compensatory hyperproliferation at later stages[25,131,132]. Likewise, HSC depletion also impaired hepatocyte proliferation after treatment with the CAR

agonist TCPOBOP[25]. Although HSCs express most of the liver's HGF, the regeneration-promoting effects of HSC are not solely mediated by HGF as HSC-selective deletion of HGF does not affect liver regeneration in mice following 70% partial hepatectomy[34]. This lack of effects might be due to HGF being stored in the liver's ECM and the possibility that endothelial cell-derived HGF, even if quantitatively less than HSC-derived HGF, could fill up these stores sufficiently for regeneration after 70% partial hepatectomy. Similar to genetic HSC depletion, HSC-specific deletion of *Rspo3* leads to a substantial impairment of liver regeneration following 70% partial hepatectomy or after treatment with TCPOBOP[25]. A comparable phenotype as in HSC-depleted mice or mice with HSC-selective deletion of RSPO3 is seen in mice with hepatocyte-specific deletion of β-catenin or LGR4/5, including inhibition of early stages of regeneration but hyperproliferation in later stages[46,70]. The transcriptional upregulation of its target gene, cyclin D1, seems to be a central mechanism through which the RSPO3–β-catenin pathway promotes hepatocyte proliferation and contributes to liver regeneration. Accordingly, cyclin D1 expression is strongly suppressed in mice with HSC depletion, HSC-selective deletion of RSPO3 or hepatocyte-specific deletion of β-catenin[25,33,70]. Furthermore, NTF3 might represent another HSC-derived promoter of liver regeneration as its overexpression rescued the deficient cyclin D1 expression and decreased liver mass in mice with HSC depletion[33]. FGFs have a key role in hepatocyte proliferation and liver regeneration, as shown through the hepatocyte-specific knockout of key receptors in mice, but the hepatomitogenic activity of FGFs has been ascribed to sources other than HSCs[133,134]. Besides growth factors, liver regeneration is also regulated via metabolic pathways and metabolite availability. Deficiency of LRAT, which promotes retinyl ester storage and is highly expressed in quiescent HSCs, leads to a moderate decrease in liver regeneration following partial hepatectomy in mice[135].

In summary, quiescent HSCs expressing high levels of RSPO3, HGF, NTF3 and LRAT promote liver regeneration. Notably, liver regeneration not only contributes to survival following massive acute liver injury but might also switch the balance from liver failure or fibrogenesis towards liver health in CLD[130,136]. Therefore, HSC-mediated promotion of liver regeneration seems to be a protective pathway that restores liver health in acute liver disease and CLD.

Regulation of the immune cell balance by HSCs

Similar to RSPO3 and HGF, the expression of several HSC-secreted mediators shaping the hepatic immune environment, such as BMP9 and BMP10, decreases during HSC activation and the development of liver disease[137]. The loss of BMP9 and BMP10 expression in mouse HSCs is correlated with the switch from resident Kupffer cells to bone marrow-derived macrophages, which are believed to drive key aspects of CLD while having a diminished ability to protect the liver from translocating bacteria[138,139]. This correlation is further supported by the replacement of Kupffer cells by monocyte-derived macrophages in mice with HSC-specific *Gdf2* and *Bmp10* knockout[37]. In addition to BMP9 and BMP10, HSCs are also enriched in CSF1, IL-34 (ref. 22) and several BMP family members such as BMP4, BMP5 and GDF6 (ref. 78). CSF1 has been suggested to contribute to monocyte to Kupffer cell differentiation during Kupffer cell repopulation in mice[22]. Furthermore, HSC-derived SOCS1, a negative regulator of cytokine signalling, protects mice from inflammation and liver fibrosis as revealed by increased infiltration of $Ly6C^{hi}CCR2^{+}$ pro-inflammatory macrophages and HSC activation in mice with HSC-specific SOCS1 deletion[140]. Similarly, HSC-specific deletion of CEACAM1, which mediates adhesion between neighbouring cells via homophilic and heterophilic interactions and mostly triggers inhibitory signals, increases macrophage and neutrophil recruitment and activation, accompanied by an unregulation of CCL2 and IL-6 and spontaneous liver fibrosis in mice of older age[141]. Together, these findings suggest that CEACAM1 acts as a protective signal that maintains HSC quiescence and liver immune homeostasis.

HSCs also positively and negatively regulate the activity of different T cell subsets and can thereby contribute to the immune-tolerant nature of the liver and the induction of specific T cell responses. Together with dendritic cells and TGFβ, HSCs induce functional T regulatory cells via an all-*trans*-retinoic acid-dependent mechanism[142,143]. However, HSCs have also been suggested to veto CD8 T cell activation by a CD54-dependent mechanism[144]. Moreover, HSCs might function as antigen-presenting cells[145,146], elicit antigen-specific T cells and protect from bacterial infection[146]. Additional in vivo studies with HSC-specific manipulations are needed to delineate their role in the regulation of T cell responses. Furthermore, there are additional candidate mediators that are highly expressed and/or enriched in HSCs, such as CXCL12, BMP5, SOCS3, RELN and collectin family members COLEC10 and COLEC11, which mostly decrease with HSC activation and could thereby switch the lymphocyte and myeloid cell environment from homeostatic to pathogenic. The interaction between HSCs and immune cells is bidirectional. Accordingly, natural killer cells can kill activated HSCs and thereby reduce liver fibrosis[147]. However, it is not known whether HSCs actively provide signals that promote the recruitment of natural killer cells to the fibrotic liver.

Prevention of excessive fibrosis and promotion of fibrosis resolution by HSCs

HSCs can inhibit liver fibrosis via two mechanisms, namely, by prevention of their own activation and by promotion of fibrosis resolution. To prevent erroneous HSC activation and fibrosis in the healthy liver, powerful profibrogenic mediators such as TGFβ must be tightly controlled, which is achieved by the secretion of mediators such as ECM1 (refs. 83,84). Whereas the effect of ECM1, namely, inhibiting latent TGFβ activation, has largely been ascribed to hepatocytes[83], quiescent HSCs also represent a major source of ECM1 (ref. 84) and might restrain TGFβ activation and fibrosis in early disease stages. GDF10 represent another HSC-secreted inhibitor of TGFβ signalling, and its loss exacerbates CCl_4-induced liver fibrosis[85]. There might also be negative feedback signals that are upregulated in activated HSCs during advanced fibrosis. For example, discoidin domain-containing receptor 2 (DDR2) expression is increased in HSCs from CCl_4-activated mouse livers and restrains HSC activation and liver fibrosis via paracrine interactions with macrophages[148].

In addition to inhibiting fibrogenesis, HSCs can reduce fibrosis via degradation of the ECM via matrix metalloproteinases (MMPs). Several MMPs are upregulated in activated HSCs but are counteracted by TIMP1, which inhibits MMP activity and is highly expressed in activated HSCs[149,150]. The balance between MMPs and TIMP1 is differentially affected by cytokines. TGFβ downregulates MMPs and upregulates TIMP1 whereas pro-inflammatory cytokines, such as IL-1 and TNF, upregulate MMPs and downregulate TIMP1 (ref. 151). Moreover, high stiffness also shifts the balance towards low MMP and high TIMP1 expression[152]. Thus, high MMP expression most likely occurs during regression phases and might contribute to the restoration of liver architecture. As macrophages also represent a key source of MMPs

as well as IL-1 and TNF and contribute to liver restoration[153,154], the interaction between macrophages and HSCs in achieving ECM degradation during resolution phases require further study.

Protection from liver cancer development by HSCs

Consistent with their beneficial effects on hepatocyte death, regeneration and metabolism, quiescent HSCs protect from the development of HCC. Protection from HCC is largely achieved via mediators such as HGF and RSPO3 (refs. 25,34) (Fig. 3) but it is possible that additional HSC-enriched mediators, such as BMP9 and BMP10, also protect from HCC development. HSC-expressed HGF primarily prevents hepatocyte death and the ensuing vicious cycle of inflammation, fibrosis and regeneration, which collectively promote HCC[34] (Figs. 1 and 3). HSC-expressed RSPO3 promotes hepatocyte metabolic function and protects from MASLD-associated hepatocarcinogenesis[25] but not from HCC induced by CYP450-activated carcinogens such as diethylnitrosamine (R.F.S., unpublished observations). Expression of the protective HSC mediators RSPO3 and HGF progressively decreases with HSC activation but some of this decrease might be compensated for by the increased numbers of HSCs in the fibrotic liver[34]. At the same time, the accumulation of HSCs in fibrotic septa[1,7] might leave sinusoids with fewer HSCs expressing protective mediators and, thereby, contribute to an increased risk for HCC formation in specific anatomic niches. This mechanism might be especially relevant for regenerating nodules or adenomas.

Pathogenic roles of HSCs in the diseased liver

Fibrotic diseases have been estimated to contribute to 45% of all deaths[155], and the liver is no exception, showing a central pathogenic role for fibrosis in CLD[8–12]. Among many parameters investigated, fibrosis has emerged as the primary determinant of outcomes in various liver diseases, including MASLD, and is associated with overall mortality, the development of liver-related events and HCC as well as with cardiovascular mortality[8–12]. Hence, the activation and proliferation of HSCs have been viewed as central pathogenic events in CLD.

Promotion of liver fibrosis

HSCs are best known for their contribution to the production of interstitial ECM, which exerts multiple pathofibrogenic roles in CLD. Since their original discovery by von Kupffer, HSCs have gradually moved into the centre of attention for these pathofibrogenic properties. Spatial associations between HSCs and liver fibrosis had already been made by Ito and Shibasaki using electron microscopic studies[20]. Subsequently, Kent et al. observed transitional cells with features of HSCs and fibroblasts in fibrotic septa following toxic liver injury[156]. Isolation of HSCs via gradient densitometry by Friedman et al. directly demonstrated that they constitute the hepatic cell population with the highest synthesis of total collagen, determined by hydroxyproline, in cell culture[5]. In vivo confirmation and more precise quantification of the contribution of HSCs to the pool of collagen-producing cells were achieved in transgenic mouse models and by scRNA-seq analysis. Tracing studies using *Lrat*–Cre or *Gucy1a1*–CreERT2 mice showed an up to 95% contribution of HSCs to type I collagen production in toxic and metabolic liver injury models[6,93,157]. By contrast, the results on the contribution of HSCs to type I collagen-producing cells in mouse models of biliary injury diverge between different studies[6,93,157]. Single-nucleus RNA-sequencing studies have confirmed HSCs as a primary source of pathofibrogenic cells in mouse or human liver[77,158]. However, the relative contribution of other fibroblasts, in particular portal fibroblasts, to the fibroblast pool

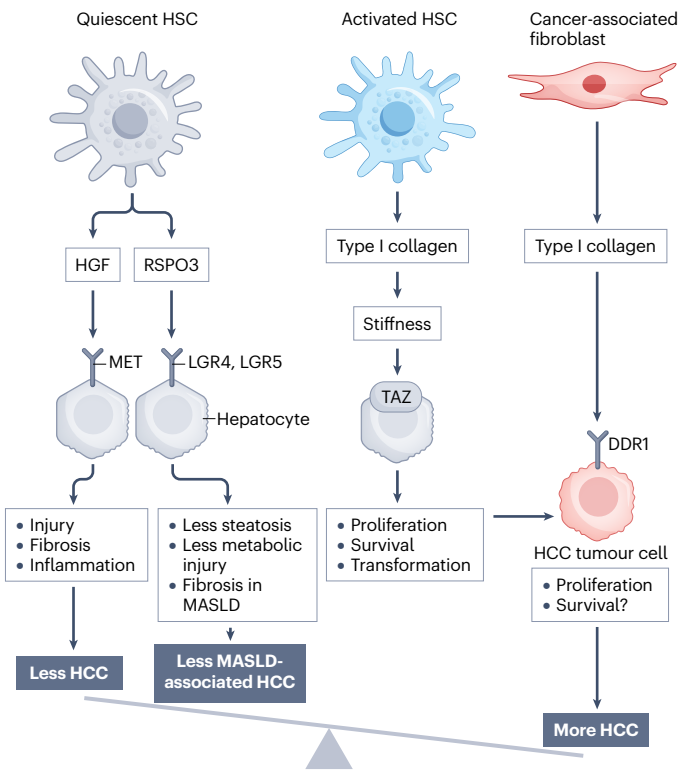

Fig. 3 | Opposing functions between quiescent and activated HSCs and their mediators in HCC. Quiescent hepatic stellate cells (HSCs) protect from the development of hepatocellular carcinoma (HCC) via hepatocyte growth factor (HGF), which prevents hepatocyte death and the ensuing inflammation, regeneration and fibrogenesis. In the setting of metabolic dysfunction-associated steatotic liver disease (MASLD), R-spondin 3 (RSPO3) secreted from quiescent HSCs protects from steatosis, liver injury, and fibrosis and reduces the risk for MASLD-associated HCC. By contrast, activated HSCs in the chronically injured liver contribute to a tumour-friendly environment via the production of type I collagen and stiffness-mediated activation of transcriptional coactivator with PDZ-binding motif (TAZ), promoting hepatocyte proliferation, dedifferentiation and transformation. Cancer-associated fibroblasts within HCC lesions produce type I collagen and might activate discoidin domain-containing receptor 1 (DDR1), promoting tumour cell proliferation and HCC progression. LGR, leucine-rich repeat-containing G-protein coupled receptor.

remains to be determined by single-nucleus RNA sequencing. Besides portal fibroblasts, fibrocytes were suggested as potential regulators or contributors to liver fibrosis[159] but have not been observed in scRNA-seq analysis of mouse models or patients. Mesothelial cells, which serve as a source for both HSCs and portal fibroblasts during development, contribute to capsular and subcapsular fibrosis[160,161]. In summary, the liver contains — in contrast to other organs — HSCs as a single and unique dominant pathofibrogenic cell population, with other cell types only making minor contributions to the production of fibrillar collagens or operating in specific settings or anatomical niches like the portal tract.

As fibrosis is considered a key driver of disease progression and determinant of outcomes in CLD, the activation process of HSCs that turns them into ECM-secreting fibrosis has been investigated in detail, with the hope of developing strategies for the therapeutic inhibition of HSC activation. TGFβ is the most potent inducer of HSC activation[7]. Its activity is regulated at multiple levels to induce activation in the

right spatiotemporal context while keeping TGFβ and HSCs in an inactive state during homeostasis[162]. TGFβ is secreted in an inactive precursor form, termed latent TGFβ, and its activation is mediated by integrins, thrombospondin and proteases as well as tensile force but inhibited by ECM1 (refs. 84,162,163). αv Integrin is expressed in HSCs themselves, rendering HSC activation a complex but potentially targetable mechanism, as latent TGFβ activation might be distinct from other cell types[83,84,162,163]. PDGF is a potent inducer of HSC proliferation and migration[164,165] and likely acts in concert with TGFβ to achieve the expansion of activated fibroblasts at, and their migration towards sites of injury, typical for the injured liver. The deposition of a provisional fibrin matrix might also be a key step in fibrosis development as suggested by decreased fibrosis in mice deficient in plasminogen activator inhibitor 1 (PAI1), which inhibits the activation of the fibrinolytic enzyme plasmin[87,166]. Moreover, PAI1 also promotes ECM degradation by increasing plasmin-mediated activation of MMPs as seen in the liver of mice with PAI1 deficiency[166]. A wide range of additional mediators, including angiotensin, leptin, IGF1 and P2Y14 ligands, have been described to contribute to HSC activation, proliferation and migration[7,167–172].

In their activated state, HSCs secrete a vast array of collagenous and non-collagenous ECM components as well as cytokines and growth factors. Fibrillar collagens, including collagens type I and III, are considered pathogenic when excessively accumulating during CLD (Fig. 4). Collagen fibrils are the primary load-bearing element in the extracellular space and likely ensure structural stability following either acute injury or repeated bouts of injury in CLD[173]. Type I collagen is the most abundant ECM protein in our body[174] and the most abundant collagen in the cirrhotic liver[101,175,176]. The majority of collagen fibrils in wound healing are, in fact, mixed, containing largely type I collagen with additional type III and V collagens[177,178]. The biology and pathobiology of fibrillar collagen remain incompletely understood, but its main effects in CLD seem to be pathogenic and mediated by both mechanical properties and signalling functions. Type I collagen is a major contributor to stiffness and activates mechanosensitive signalling pathways in hepatocytes and other hepatic cell types as demonstrated in mice with deletion of *Col1a1* (ref. 34). Crosslinking of collagen by lysyl oxidase or lysyl oxidase-like family members increases stiffness in liver fibrosis as shown in preclinical models by broad-spectrum pharmacological inhibition via β-aminoproprionitrile[179,180]. Changes in collagen-mediated stiffness exert effects on many hepatic cell types, which are primarily mediated via integrin-containing focal adhesions that link the extracellular environment to intracellular signalling machinery and trigger the activation of mechanosensitive signalling pathways, including YAP–TAZ, FAK–SRC, PI3K–AKT and RHOA–ROCK[181]. In hepatocytes, consequences of stiffness-induced signalling include the reduced expression of HNF4α, a transcription factor with key roles in hepatocyte identity and function[182], via an RHOA–ROCK-mediated pathway[183] (Fig. 4). Furthermore, collagen-induced stiffness activates TAZ and YAP in hepatocytes, which might not only affect hepatocyte proliferation, differentiation and resistance to cell death but also the secretion of profibrogenic mediators[34,184,185]. In addition, collagen-mediated

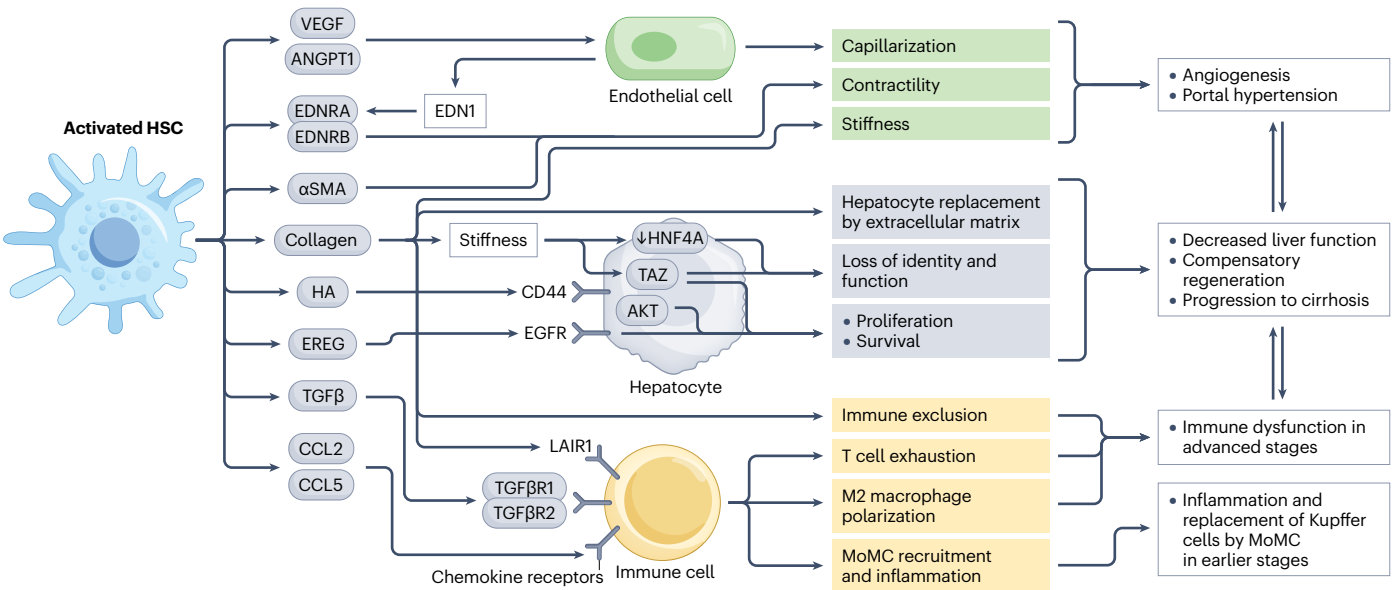

Fig. 4 | Pathogenic roles of HSCs during fibrogenesis. In the chronically injured and fibrotic liver, activated α-smooth muscle actin (αSMA)-positive hepatic stellate cells (HSCs) and their interactions with endothelial cells contribute to portal hypertension through multiple mechanisms, which include contractile properties of αSMA$^+$ HSCs, triggered via endothelial cell-secreted endothelin 1 (EDN1) and high expression of endothelin receptors EDNRA and EDNRB on HSCs; the production of collagens and the resulting increase in liver stiffness; and increased angiogenesis mediated by vascular endothelial growth factor (VEGF) and angiopoietin 1 (ANGPT1). Collagen production by activated HSCs contributes to liver stiffness and subsequent activation of mechanosensitive signals such as transcriptional coactivator with PDZ-binding motif (TAZ); hyaluronic acid (HA) production by activated HSCs triggers AKT activation in hepatocytes via CD44. Together, these signals promote hepatocyte proliferation and survival, which help to maintain functional liver mass and contribute to the formation of regenerative nodules but might also drive hepatocyte transformation in the long term. Collagen accumulation, stiffness and TAZ activation might also induce a loss of hepatocyte identity and replacement of functional liver mass by extracellular matrix. HSC-derived chemokines promote the recruitment of monocyte-derived macrophages (MoMC) and inflammation in early disease stages; transforming growth factor-β (TGFβ) might induce T cell exhaustion and M2 macrophage polarization in advanced stages. Furthermore, HSC-derived collagen might provide a physical barrier, leading to immune cell exclusion, and activate immunosuppressive collagen receptors such as leukocyte-associated immunoglobulin-like receptor 1 (LAIR1) in advanced disease. CCL, CC-chemokine ligand; EGFR, epidermal growth factor receptor; EREG, proepiregulin; HNF4α, hepatocyte nuclear factor 4α; TGFβR, TGFβ receptor.

stiffness results in the activation of FAK, which increases the resistance to cell death[186]. Increased stiffness also affects other cell types such as endothelial cells and HSCs. In liver sinusoidal endothelial cells, increased stiffness conditions reduce the number of fenestrations[187], a feature termed 'capillarization', which occurs during liver fibrosis development and can exacerbate liver disease progression (Fig. 4). HSCs are also strongly affected by stiffness in vitro, resulting in increased activation[188]. By contrast, reduced stiffness via the deletion of type I collagen, determined by HSC-specific *Col1a1* knockout, does not seem to impair HSC activation in mice in vivo[34]. In addition to alterations of mechanical properties and mechanosensitive signalling pathways, collagens and collagen cleavage products activate a wide range of receptors on resident liver cells and immune cells. DDR1 is a receptor for fibrillar collagens and is predominantly expressed on cholangiocytes and dendritic cells, where its role remains to be determined. Hepatocellular DDR1 also becomes upregulated in hepatocytes and is mainly implicated in hepatocarcinogenesis[34]. Furthermore, hepatocellular DDR1 has also been implicated in hepatic fibrogenesis by some but not other studies[34,189]. In other organs, such as the breast, DDR1 has a role in the reorganization of collagen, and its deletion — in contrast to the liver — increases fibrosis and promotes immune exclusion and, thereby, tumour development[190,191]. Besides DDR1, integrins represent another group of collagen receptors that often recognize the Arg–Gly–Asp (RGD) sequence of collagens, providing the cells with key information about the surrounding ECM and turning on a wide range of intracellular signalling pathways in response. Additional receptors for collagens, including LAIR1, OSCAR and GPR56, show only low expression in HSCs (R.F.S., unpublished observations) and their role in CLD requires further study. In summary, HSC-derived collagen expression seems to promote CLD progression by furthering the loss of hepatocyte functions and stiffness-induced secretion of profibrogenic mediators from hepatocytes, as well as endothelial capillarization (Fig. 4).

Along with other cell types, such as endothelial cells, HSCs also contribute to the production of non-fibrillar collagens and other ECM components. The production of basement membrane ECM components, such as type IV collagen, is strongly upregulated in activated HSCs[192], but it remains unclear whether this type of HSC-derived ECM serves protective or pathogenic functions in CLD. Moreover, the majority of basement type IV collagen is produced by endothelial cells and seems to promote portal hypertension in CLD[193]. Cleavage products from collagen type I, IV, VI, VIII, XV and XVIII, generated by MMPs, provide feedback signals to multiple liver cell types, including endothelial cells and HSCs, through a wide range of receptors and mechanisms (reviewed previously[177]). Beyond collagens, many other ECM components are predominantly secreted by activated HSCs, such as hyaluronic acid, tenascin (TNC), decorin, periostin, lumican and some laminins, and shape the fibrotic niche, contributing to the specific mechanical properties of the fibrotic liver and the activation of specific signalling pathways[194] (Fig. 3). A disease-promoting role for HSC-produced hyaluronic acid has been described for the development of toxic, biliary and metabolic liver fibrosis, in which it amplifies the activation of HSCs[195]. TNC, primarily expressed in HSCs, promotes the development of concanavalin A-induced liver fibrosis as shown in mice with global *Tnc* knockout[196,197]. Periostin seems to be produced mainly by HSC and to promote their activation via TGFβ and lysyl oxidase-mediated pathways[198]. Liver biology and pathobiology are not only regulated by the ECM itself but also through molecules bound to the ECM, collectively known as the 'matrisome' (reviewed previously[199,200]). In addition to many ECM components, HSCs also secrete matrisome ingredients, including HGF, ECM1 and ANGPTL2, which can be released from the ECM following injury and ECM cleavage[199,200]. In summary, changes in the hepatic ECM, induced by the activation of HSCs, include not only fibrillar collagens but also non-collagenous ECM and other matrisome components as well as ECM and matrisome receptors and mechanosensory systems. Together, these systems regulate hepatocyte injury, proliferation, and liver function and contribute to the switch towards an environment that contributes to liver disease progression and the development of liver cancer[194,199,200].

Although the change in ECM and matrisome, commonly simplified as 'fibrogenesis' or 'fibrosis', might be beneficial in some contexts, for example, by providing mechanical stability, enhancing regeneration and restoration of liver function, further studies are needed to understand the benefits versus the harms of this complex system in a context-dependent and temporospatial manner. It is likely that fibrogenesis is geared towards acute and potentially fatal liver diseases such as massive toxic injury, mechanical injury or acute infection. By contrast, the chronic activation of HSCs, common to diseases that have become frequent in modern societies such as MASLD or chronic viral hepatitis, are maladaptive and might not have been selected against during evolution as they rarely affect reproductive fitness due to slow progression of most forms of CLD and the development of severe symptoms only in advanced age.

Promotion of angiogenesis and portal hypertension

Angiogenesis is a central component of nearly all wound healing processes. In the liver, angiogenesis is closely associated with fibrosis and cirrhosis[201,202]. Beyond a mere association, angiogenesis has been proposed to sustain fibrogenesis and drive the development of HCC[201]. Angiogenesis in the fibrotic liver is promoted by activated HSCs, involving the secretion of VEGF and angiopoietin 1 via HIF1A, TGFβ and Hedgehog-dependent signalling pathways[203–208].

Portal hypertension is another key feature of advanced liver disease and contributes to its complications[41]. Portal hypertension is linked to several features associated with HSC activation, including increased contractility, fibrogenesis and secretion of angiogenic mediators. Via their long cytoplasmic process and contractile machinery, HSCs can regulate the liver's vascular tone[40,209,210]. Importantly, HSC activation increases the expression of α-smooth muscle actin (αSMA; encoded by *ACTA2*) and enhances the contractility of HSCs[211,212]. In the fibrotic liver, the increased expression of αSMA as well as alterations of HSC–endothelial interactions, such as decreased production of the vasodilator nitric oxide and high expression of endothelin receptors on HSCs with concomitant increases in the production of the vasoconstrictor endothelin 1, contribute to the development of portal hypertension[40,41,209,213–215] (Fig. 4). The accumulation of ECM not only leads to a replacement of functional liver parenchyma but also modifies the sinusoidal milieu and, thereby, vascular resistance of the liver[40]. Furthermore, angiogenesis contributes to the development of portal hypertension by promoting portosystemic collaterals and splanchnic vasodilatation[216]. Accordingly, the production of VEGF by HSCs leads to endothelial cell capillarization and thereby worsens portal hypertension[41]. A study published in 2024 demonstrated that HSC-expressed protocadherin 7 represents a mediator that regulates sinusoidal contraction in mice in response to endothelin 1 (ref. 217).

Regulation of hepatic inflammation

There is a bidirectional crosstalk between HSCs and immune cells. Immune cells contribute to the activation of HSCs by providing TGFβ and other profibrogenic mediators[7,13,218]. At the same time, activated

HSCs are thought to contribute to the recruitment and activation of immune cells in the fibrotic liver[218–220]. As such, HSCs secrete a wide range of pro-inflammatory mediators implicated in the development of liver fibrosis, including CCL2, CCL5 and CXCL12 (refs. 218,221–224). Moreover, it has been suggested that, via the expression of VCAM and ICAM1, HSCs promote the adhesion of immune cells and, via HGF, IL-6 and VEGF, their migration[225]. The altered expression of HSC-derived BMP9, BMP10, CSF1 and IL-34 in activated HSCs within the fibrotic liver might contribute to changes in the macrophage niche[22]. Furthermore, in vitro studies show that HSC-derived signalling molecules promote the differentiation of liver macrophages with pro-inflammatory and profibrotic functions in a p38-dependent manner[226]. However, current scRNA-seq data, for example, from the LiverCell Atlas[78], suggest that the majority of inflammatory mediators, including CCL2 and CCL5, are much more abundantly expressed in other cell types than in HSCs. Moreover, most studies investigating inflammatory cytokine expression and HSC-mediated leukocyte adhesion, infiltration and activation were conducted in vitro or using whole-body knockout mice[218,220]. Interestingly, a study achieving HSC-selective hyperactivation of the NLRP3 inflammasome in *Lrat*–Cre mice found an increase in HSC activation and liver fibrosis but no alterations in inflammation or injury, suggesting a cell-autonomous role for NLRP3 in HSCs and a disconnect between the HSC inflammasome and hepatic inflammation[227]. Hence, in vivo validation with HSC-selective knockout mice is needed to firmly establish the role of HSCs in inflammation and to reveal the function of specific HSC candidate mediators in regulating cellular networks and hepatic inflammation. Consistent with the bidirectional interaction between activated HSCs and immune cells in CLD, HSC activation and survival are regulated by specific mediators secreted from macrophages, T cells and regulatory T cells, including IL-1β, TNF and amphiregulin[13,14,228,229].

Promotion of liver cancer growth by activated HSCs

HCC and cholangiocarcinoma (CCA) represent the most common primary liver cancers. In addition, the liver is one of the most common and often first sites of metastasis, for example, in pancreatic cancer[36,230–232]. Although HSCs do not have a known role in cellular transformation, they can provide an environment that favours the development and growth of liver cancer[233,234]. The overall tumour-promoting role of HSCs has been shown by genetic depletion in mouse models of HCC and CCA as well as in colorectal and pancreatic liver metastasis[34–36]. HSCs exert distinct and sometimes opposing roles in liver cancer development, similar to CLD[25,34,233]. Moreover, there are substantial differences between these tumour entities, with several HSC mediators, such as HGF and type I collagen, exerting distinct and sometimes opposing effects on HCC and CCA[233]. This discrepancy might be linked to the finding that HCCs develop in fibrotic livers but that HCCs often contain fewer HCCs than the surrounding fibrotic tissue, whereas desmoplastic CCA can even develop in non-fibrotic livers but is usually highly desmoplastic with abundant HSC-derived cancer-associated fibroblasts[34,35,233,235]. For deeper insights into the role of HSCs in the growth of desmoplastic liver tumours, including CCA and their differences to HCC, we refer to reviews of this topic[35,233,234].

Consistent with the diverse and stage-specific functions of HSCs and their mediators in CLD, HSCs can either protect from or promote HCC[34]. Collagen-producing HSCs contribute to a stiff environment in premalignant stages, leading to the activation of TAZ in hepatocytes and the subsequent development of HCC[34], as determined by the HSC-specific knockout of *Col1a1* and hepatocyte-specific knockout of *Taz* in mice (Fig. 3). The main effects of the type I collagen–TAZ signalling axis in hepatocarcinogenesis seem to be via hepatocyte proliferation and survival[34]. Although less abundant, HSC-derived cancer-associated fibroblasts within HCCs are highly activated and additionally promote HCC development in mice by producing type I collagen and activating the collagen receptor DDR1 and DDR1-mediated proliferation in tumour cells[34] (Fig. 3). Besides type I collagen, HSCs contribute to HCC development by producing hyaluronic acid via hyaluronan synthase 2 (ref. 34). In addition to ECM components, HSC-secreted growth factors, such as epiregulin, might promote hepatocyte proliferation and development of HCC[236,237]. Epiregulin expression is upregulated in activated HSCs and further increases after stimulation with lipopolysaccharide[237]. Whereas expression levels of HSC mediators protecting from HCC, such as RSPO3 and HGF, decrease with HSC activation, type I collagen and hyaluronan synthase 2 expression levels increase, suggesting that the balance of HSCs shifts from protection to HCC promotion during CLD progression. *LRAT* is a notable exception as it represents an HSC quiescence gene that seems to promote hepatocarcinogenesis[92,99,238]. However, it is possible that these effects, established in a global *Lrat*-knockout mouse model, are mediated by LRAT-expressing cell populations other than HSCs. HSCs also secrete a wide range of additional mediators that have been associated with the promotion of hepatocarcinogenesis, including TGFβ, IL-6, CLCF1, GDF15, VEGF, TNC, laminins and fibronectin (reviewed previously[233,235]). Activated HSCs can contribute to a tumour-promoting immune cell dysfunction by ECM-mediated physical exclusion or by altered expression of immunosuppressive mediators, including TGFβ, PDL1 and macrophage migration inhibitor factors, which have been reviewed in detail in other reviews[194,233,234].

Implications of HSC balance for liver disease and its treatment

Current studies suggest that the shifting balance between protective and pathogenic HSC mediators constitutes a central parameter determining outcomes in CLD[25,34]. Understanding mechanisms that change this balance during liver disease progression and regression and how it can be therapeutically restored might open up novel treatment approaches.

A shift from HSC-mediated protection to HSC-mediated disease promotion in CLD

The conversion from retinoid-storing pericyte-like quiescent HSCs, dispersed throughout the liver lobule, to ECM-producing activated HSCs, predominantly located in fibrotic septa, is accompanied by a massive change in gene expression and functions, affecting their communication with other liver cells and exerting profound effects on liver disease development and progression[7,192,239]. Traditionally, the focus has been mostly on understanding the HSC activation process and targeting the pathofibrogenic features of HSCs. However, with the discovery of hepatoprotective pathways in HSCs, it appears that the balance between hepatoprotective and pathogenic mediators in HSCs determines their role in disease progression (Fig. 5). Factors that mediate the hepatoprotective functions of HSCs, such as HGF, RSPO3, BMP9, BMP10 and possibly other HSC-enriched mediators, decrease with the progression of CLD in mice and patients[25,34,37] (Fig. 5). The reduction of protective HSC mediators is associated with increased disease progression in mouse models and, for RSPO3, also with mortality and liver-related events in patients[25,34]. As these mediators activate distinct pathways and biological effects in their target cells, they likely exert additive or synergistic protective effects, with low expression of

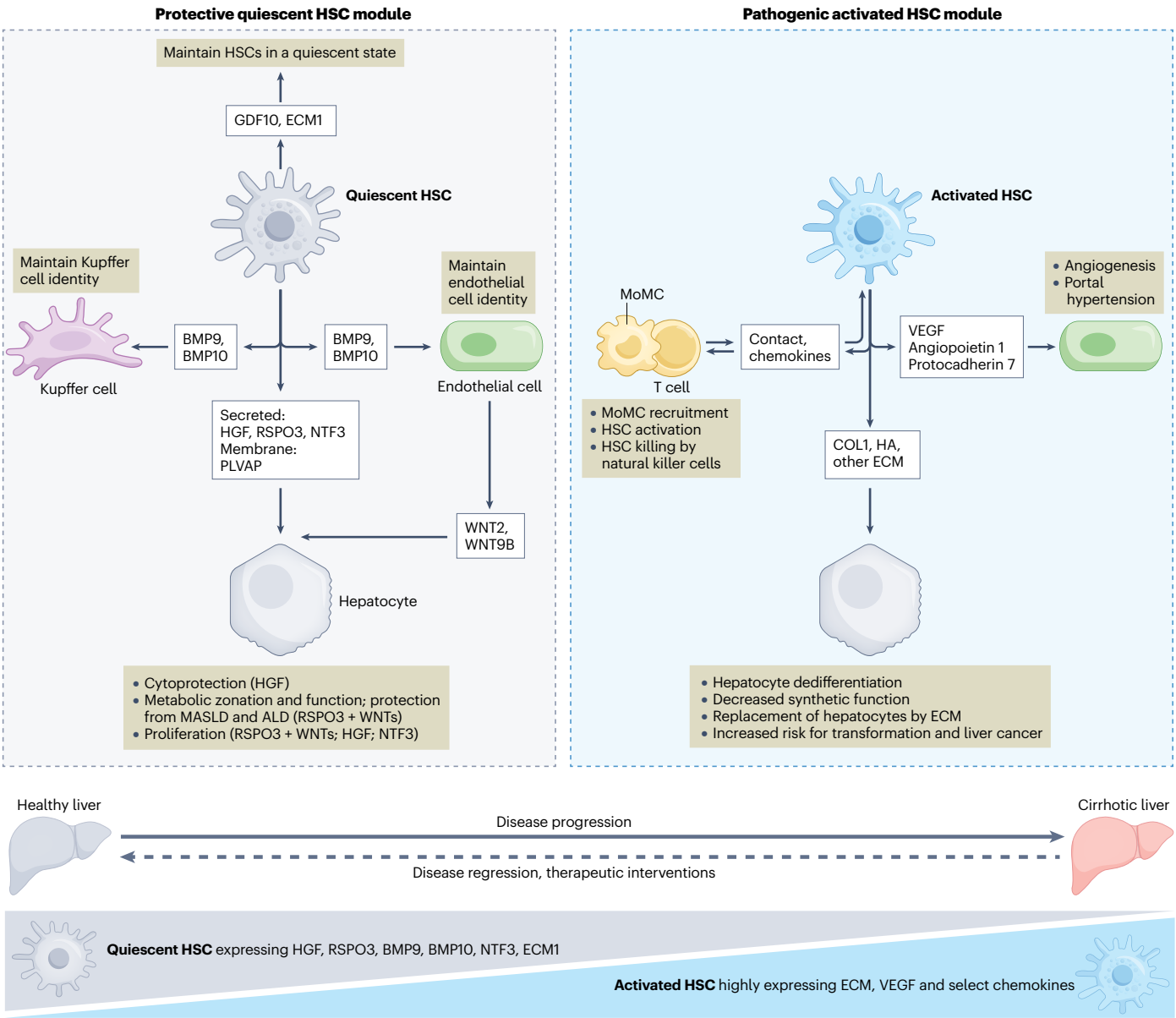

Fig. 5 | A shifting balance between protective and pathogenic HSC mediators contributes to the progression of chronic liver disease. During the progression of chronic liver disease to fibrosis, cirrhosis and hepatocellular carcinoma, hepatic stellate cells (HSCs) become activated and lose their hepatoprotective properties (via R-spondin 3 (RSPO3), hepatocyte growth factor (HGF), bone morphogenetic protein 9 (BMP9), BMP10, plasmalemma vesicle-associated protein (PLVAP)) while increasingly expressing pathogenic mediators (type I collagen (COL1), hyaluronic acid (HA), vascular endothelial growth factor (VEGF) and chemokines). Quiescent HSCs and activated HSCs do not act on their own but in multicellular modules. Both the protective quiescent HSC module and the pathogenic activated HSC module include endothelial cells, Kupffer cells or bone marrow-derived inflammatory cells and hepatocytes. Restoration of this balance towards protective mediators occurs during fibrosis resolution and might also represent a novel approach for the treatment of chronic liver disease that not only inhibits fibrogenesis but also restores homeostasis and liver health. Autocrine loops involving growth/differentiation factor 10 (GDF10) and extracellular matrix protein 1 (ECM1) may keep HSCs in a quiescent state. ALD, alcohol-associated liver disease; ECM, extracellular matrix; MASLD, metabolic dysfunction-associated steatotic liver disease; MoMC, monocyte-derived macrophages; NTF3, neurotrophin 3.

multiple protective HSC mediators in advanced liver disease potentially causing detrimental effects. At the same time, HSC-derived pathogenic mediators, such as type I collagen, hyaluronic acid, TNC and periostin, might also exert additive or synergistic pathogenic effects, contributing to fibrosis progression, portal hypertension and an increased risk for HCC development. Together, this suggests that the balance between the aggregate of protective mediators versus the aggregate of pathogenic mediators determines the effects of HSCs on liver disease progression (Fig. 5). Moreover, HSC-derived hepatoprotective mediators might be part of feedforward circuits that stabilize the protective

state of HSCs. For example, HSC-derived HGF decreases hepatocyte death in mice, thereby limiting cell death-induced HSC activation and keeping the balance towards healthy HSCs with high expression of other protective mediators[34]. Likewise, RSPO3 decreases ALD-induced and MASLD-induced steatosis and liver injury in mice, thereby indirectly protecting HSCs from activation[25]. In CLD, the expanded pool of HSCs might, in part, compensate for the relatively lower expression of protective mediators within each single HSC, thereby providing continuous protection even in CLD. At the same time, the spatial expression of protective mediators might be altered when HSCs migrate and accumulate in fibrotic septa, leaving other areas potentially with fewer HSC-expressed protective mediators. Whether positive feedforward or negative feedback mechanisms exist for pathogenic mediators in activated HSCs, such as type I collagen and other ECM components, remains largely unknown. The high expression of collagen receptor DDR2 on HSCs and the finding that global DDR2 deficiency increases CCl_4-induced liver fibrosis in mice[148] suggest the possibility of negative feedback mechanisms for collagens. Indeed, circuitries that stabilize the protective quiescent HSC state rather than the activated HSC state would be beneficial as they would favour the return to liver health and it is possible that HSC-expressed ECM1 and GDF10 are components of such circuits (Fig. 5).

Further studies are needed to identify and functionally assess additional hepatoprotective and pathogenic HSC mediators as the effects of many ligand–receptor interactions of this highly interactive cell type on the progression of liver disease remain unknown. Moreover, the interaction of HSCs with cell types like cholangiocytes and specific immune cell subsets such as macrophages and dendritic cell subtypes, and the underlying mediators and mechanisms deserve additional study and can further expand our knowledge on the protective and disease-promoting effects of HSCs. Although mechanisms regulating the transcription of pathogenic mediators, such as type I collagen, have been studied in detail, there is limited information on how protective mediators are regulated in HSCs. As TGFβ not only upregulates pathogenic HSC mediators like type I collagen and lysyl oxidase but also downregulates the expression levels of protective mediators such as HGF and RSPO3 (ref. 25), it is likely that distinct pathways or transcriptional master regulators govern the balance between hepatoprotection and disease promotion in HSCs.

HSC deactivation as a naturally occurring mechanism to restore HSC balance

During fibrosis resolution, activated HSCs undergo programmed cell death[240], and surviving HSCs return to a quiescent state in a process termed HSC inactivation or deactivation[241–243]. Notably, the deactivation of HSCs encompasses the restored expression of hepatoprotective mediators such as RSPO3 and HGF[25] and possibly other protective HSC mediators in addition to a decrease in fibrogenic gene expression (Fig. 5). This process likely aids in restoring normal liver function and architecture after the underlying liver injury is eliminated. Although the deactivation process occurs rapidly in mice after the cessation of liver injury, it seems that this process does not work as quickly and efficiently in patients with advanced liver fibrosis after decades of CLD[243–246]. Thus, the therapeutic promotion of HSC deactivation might represent a powerful tool to accelerate the typically slow restoration towards beneficial HSCs and liver health in patients (Fig. 5). At the same time, deactivation occurs only in a minority of HSCs, likely because the expanded pool of activated HSCs in fibrotic livers needs to be reduced to the numbers found in a healthy liver via the induction of cell death[241–243]. Thus, combining the restoration of HSC balance towards hepatoprotection with a reduction of HSC numbers via cell death induction might represent the most promising therapeutic strategy to restore liver health.

Restoration of HSC balance as a therapeutic goal

Although there are currently no FDA-approved antifibrogenic drugs that directly reduce liver fibrosis, there is increasing progress in targeting mechanisms in hepatocytes that drive the development of liver fibrosis[247]. For example, the FDA-approved thyroid receptor agonist resmetirom stimulates hepatocyte metabolism in patients with MASLD, resulting in lowered low-density lipoprotein cholesterol, and leads to the resolution of metabolic dysfunction-associated steatohepatitis and fibrosis improvement in 24–26% of patients versus 14% in the placebo group[248]. FGF21 agonists increase insulin sensitivity and fatty acid β-oxidation, decrease lipogenesis, and achieve metabolic dysfunction-associated steatohepatitis resolution with accompanying fibrosis improvement in up to 41% of treated patients ($n = 85$) versus 20% in the placebo group ($n = 43$)[249,250]. However, specific targeting of HSCs might be additionally needed[251] as drugs like resmetirom or FGF21 agonists only work in a subset of patients[248,250] and probably not as effectively in advanced stages, in which the underlying metabolic drivers are less active[247]. Similarly, eradication of hepatitis C virus does not lead to regression of liver fibrosis or cirrhosis in some patients and combination or sequential therapies that additionally target HSCs might be needed[252].

The beneficial effects of HSCs on hepatocyte metabolism and health suggest that therapeutically shifting the HSC balance from fibrogenesis towards hepatoprotection might not only reduce the sequelae of fibrosis but also aid in restoring liver function. Combining drugs that kill HSCs (for example, BMS-986263)[253] with drugs that shift HSC balance towards protection would mimic the parallel occurrence of HSC deactivation and HSC removal by apoptosis during fibrosis regression discussed in the previous section. Future studies and drug development need to shift the focus from identifying drugs that selectively inhibit ECM production or kill HSCs towards drugs that shift the balance to achieve decreased ECM production with additional restoration of hepatoprotective mediators such as RSPO3, HGF, BMP9 and BMP10. It will be important to determine in preclinical studies if this restoration ameliorates hepatocyte and liver function and improves long-term survival. Analysis of the transcriptional machinery that controls the balance between protective and pathogenic mediators in HSCs might uncover novel therapeutic targets and treatment concepts. TGFβ seems to be a master regulator of HSC balance, increasing fibrogenic genes while reducing hepatoprotective mediators such as RSPO3 and HGF[25]. However, TGFβ and its receptors are considered difficult therapeutic targets due to strong adverse effects on the immune system[254]. Therefore, additional efforts are needed to uncover pathways that regulate HSC balance. Further analyses of HSC mediators and testing their biological functions via HSC-selective knockout mouse models might identify additional candidates through which HSCs maintain overall liver health. Importantly, drugs that restore HSC balance may provide us with much-needed weapons in our therapeutic arsenal for end-stage liver disease. Current preclinical studies demonstrated the elimination of pathogenic HSC subtypes, such as senescent HSCs, marked by PAI1 expression via chimeric antigen receptor T cells[255]. Eliminating HSCs that cannot revert to an inactive phenotype, such as senescent HSCs, might be beneficial. By contrast, the complete elimination of HSCs that are activated but have the potential to revert to an

inactive phenotype might be harmful. Besides drugs, which often act on many cell types due to the widespread expression of therapeutic targets, HSC-specific delivery might represent a promising strategy for delivering drugs that can shift HSC functions. Vitamin A-coupled lipoid nanoparticles containing di-retinamide-PEG-di-retinamide, which binds with high affinity to retinol-binding protein, might deliver cargo, such as small interfering RNA or small activating RNA, selectively to HSCs and have been used in clinical trials[253,256]. RNA delivery strategies could be harnessed to restore the HSC balance in patients with CLD by simultaneously inhibiting the expression levels of pathofibrogenic mediators while increasing the expression levels of protective mediators. HSCs exist in multiple subpopulations with distinct but often overlapping markers and only a few well-defined surface markers[19,257,258]. Defining surface markers on key populations, for example, in HSCs with high potential to revert, might enable selective delivery of drugs or RNA to these subpopulations to enhance their restorative and protective properties and should be a focus of future studies. HSC-targeted delivery strategies seem to be most attractive for advanced disease stages in which HSCs are strongly activated and hepatocyte-directed treatments have lower rates of success. Alternatively, one could combine purely antifibrotic strategies with the pharmacologic restoration of HSC-protective signals, for example, HGF mimetics or MET-agonistic antibodies[259] and RSPO3 or WNT agonists[260–262] or targeted delivery of drugs or RNAs to HSCs that shift the HSC balance towards protection.

Conclusions

The liver fibrosis field has long focused on the pathofibrogenic properties of HSCs and the therapeutic reduction of ECM accumulation whereas concepts for restoring epithelial cell health, liver function and architecture have received little attention. Further understanding of the protective properties of HSCs and HSC-derived mediators is needed to design rational and holistic therapies that restore HSC balance with the goal of converting pathogenic HSCs into protective HSCs. Due to their high prevalence and public health relevance, advanced MASLD and eradicated hepatitis C virus with remaining liver cirrhosis represent diseases in which HSC restorative therapies could be particularly beneficial. Along this line, there needs to be a better understanding of hepatic cell–cell interactions in the context of liver fibrosis as antifibrotic therapies targeting HSCs likely exert effects on liver health by affecting HSC interaction with hepatocytes, endothelial and Kupffer cells. This complex cellular crosstalk will provide novel therapeutic opportunities to restore liver function and architecture but could also contribute to adverse effects of antifibrotic therapies that eliminate HSCs rather than restoring them.

Published online: 22 May 2025

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Acknowledgements

This Review is in memory of Professor Kenjiro Wake, who made invaluable contributions to the field through his rigorous research and art that depicted key anatomical connections and regulatory functions of hepatic stellate cells. This work was supported by National Institutes of Health grants 5R01DK128955, 5R01CA262424 (all to R.F.S.), 5U01CA274295 and the Columbia University Digestive and Liver Disease Research Center 5P30DK132710, National Institutes of Health grants 5R01DK099205, R01CA285997, P50AA011999 (to D.A.B.) and the San Diego Digestive Diseases Research Center Grant 2P30DK120515.

Author contributions

R.F.S. researched data for the article. All authors contributed substantially to discussion of the content. R.F.S. wrote the article. All authors reviewed and/or edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature Reviews Gastroenterology & Hepatology* thanks Xiongwen Lv, Steven Dooley, Ramon Bataller and Sai Wang for their contribution to the peer review of this work.

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